Journal of Surgical Oncology 1998;67:90–98

Effects of en Bloc Esophagectomy on Nutritional and Immune Status in Patients With Esophageal Carcinoma

LIANG-SHUN WANG, MD,[1]* HSIAO-YI LIN, MD,[2] CHUN-JU CHANG, BS,[1] HUEI-JYH FAHN, MD,[1] MIN-HSIUNG HUANG, MD,[1] AND CHIEN-FU JEFF LIN, MD, PhD[3]

[1]*Division of Thoracic Surgery, Department of Surgery, Veterans General Hospital and National Yang-Ming Medical University, Taipei, Taiwan, Republic of China*

[2]*Department of Allergy Immunology and Rheumatology, Veterans General Hospital and National Yang-Ming Medical University, Taipei, Taiwan, Republic of China*

[3]*Department of Biostatistics, Veterans General Hospital and National Yang-Ming Medical University, Taipei, Taiwan, Republic of China*

Background and Objectives: En bloc esophagectomy has been established as the treatment of choice for patients with resectable esophageal carcinoma. However, an extensive surgical procedure may result in further impairment of the patient's nutritional status and immune system. Thus a prospective study was undertaken to evaluate the perioperative sequential changes in patients' nutritional and immune status and the timing to institute postoperative adjuvant therapy.

Methods: Thirty-seven patients (34 male, 3 female) who had undergone en bloc esophagectomy with gastric institution for epidermoid carcinoma of the esophagus were studied. The mean age was 62.3 years. The nutritional and immune assessments were performed preoperatively, on the third postoperative day, in the first week, second week, third week, and at the end of the first and third month. The biochemical studies for nutritional evaluation included serum albumin, cholesterol, iron, transferrin, magnesium, zinc, total iron binding capacity (TIBC), and nitrogen balance. Evaluation of the immune status consisted of: (1) total lymphocyte count, (2) lymphocyte subpopulation, (3) immunoglobulins, (4) complements (C3 and C4), (5) lymphocyte blastogenic responses, (6) tumor necrosis factor-α and interleukin-2 secretion activity from mononuclear cells, and (7) C-reactive protein (CRP) level.

Results: All the parameters in nutritional assessment declined profoundly by the third postoperative day ($P < 0.05$). The most severe deterioration was in serum iron, followed by transferrin, TIBC, cholesterol, and zinc. Most of them returned to the preoperative levels within 2–3 weeks after surgery. However, the serum levels of iron, transferrin, and TIBC required a longer period of time (>1 month) to return to normal. A remarkable increase of serum CRP was detected in the first postoperative week ($P < 0.05$), but immunoglobulins and complements decreased significantly yet variably ($P < 0.05$) in the second or third postoperative week before gradually returning to preoperative levels. Moreover, during the first week

Contract grant sponsor: NCS; Contract grant number: NSC82-0412-B-075-060; Contract grant sponsor: Loong-Jen Cultural and Public Welfare Foundation, ROC.

*Correspondence to: Liang-Shun Wang, M.D., Associate Professor of Surgery, National Yang-Ming Medical College, Division of Thoracic Surgery, Department of Surgery, Veterans General Hospital-Taipei, N0.201, Sec. 2, Shih-pai Road, Shih-pai, Taipei, Taiwan 11217, Republic of China. Fax No.: (886)2-873-1488.

Accepted 29 October 1997

after surgery, CD3 and CD8 diminished following esophageal surgery, whereas CD20, CD4/CD8 ratio, and lymphocyte blastogenic responses increased significantly ($P < 0.05$).

Conclusions: Except for iron-related parameters, all the other nutritional parameters returned to the preoperative level by the third postoperative week. An adequate supplementation of iron and protein for 1–3 months after surgery is needed. En bloc esophagectomy might have only a mild and temporarily adverse effect on the host immune defense. Regarding the postoperative recovery of a patient's nutritional and immune status, postoperative chemo-radiotherapy is optimally instituted after the third postoperative week, instead of within 2 weeks of surgery.

J. Surg. Oncol. 1998;67:90–98.

KEY WORDS: esophageal carcinoma; esophagectomy; nutrition and immune status

INTRODUCTION

Carcinoma of the esophagus is not only a lethal malignancy, but also one of the most distressing diseases that occurs with unfortunate frequency among the Chinese population [1–3]. The survival rate has not improved appreciably during the past 25 years despite multiple treatment modalities. Recently, en bloc esophagectomy has been advocated as the management of choice for patients who may have potentially curable esophageal carcinoma [4–7]. A systematic review of the literature has demonstrated it as being able significantly to improve prognosis of esophageal carcinoma [8–10], probably due to more accurate tumor staging and superior locoregional control of the disease by a complete resection. However, an extensive radical resection can result in a relatively higher morbidity and mortality, which may be partially due to the surgical effects on the patient's nutrition and immune status [11,12].

Although it is predicted that en bloc esophagectomy with radical lymph node dissection might worsen the patient's nutritional and immune status, the degree of deterioration and the timing for recovery have not yet been clearly defined. Thus a prospective study was conducted to detect the sequential changes in the patient's nutritional and immune status before and after en bloc esophagectomy with lymph node extirpation for epidermoid carcinoma of the esophagus so that timing for instituting postoperative adjuvant chemoradiotherapy could be optimally determined.

MATERIALS AND METHODS

Patients and Tumor Staging

From August 1992 to September 1994, 43 patients with previously untreated epidermoid carcinoma of the esophagus were originally contacted. Six patients refused a surgical operation, leaving a total of 37 patients included for study. One of the patients died of surgical complications on the fifth postoperative day. The criteria selection of patients undergoing en bloc esophagectomy included: (1) age <75 years, (2) possible cure of the localized or regional disease, (3) serum-albumin level $\geqslant$3.5 mg/dl, (4) no cigarette smoking for >3 weeks before surgery, and (5) no association with severe systemic diseases, e.g., recent myocardial infarction, or cerebral vascular accidents (<6 months), uncontrollable diabetes mellitus or hypertension, or severe chronic obstructive pulmonary diseases. The ages of the 37 patients ranged from 56 years to 73 years, with a mean of 62.3 years. Thirty-four patients were male and three were female. All had Karnofsky performance scores >90 and underwent en bloc esophagectomy with locoregional lymphadenectomy through a right thoracotomy and laparotomy with reconstruction using the stomach through a retrosternal route, and cervical esophagogastrostomy. The locoregional lymphadenectomy included two-field lymph node dissection (mediastinal and abdominal regions) for the lower third esophageal carcinomas and three-field lymph node dissection (cervical, mediastinal, and abdominal regions) for the upper two-third esophageal carcinomas [9,13]. Feeding jejunostomy was routinely performed to facilitate enteral feeding from the fifth postoperative day. All surgical operations were performed by the same surgical team. In general, oral feeding was initiated on the tenth postoperative day if there was no leakage of the cervical anastomosis. In addition, intravenous infusion of 25% albumin (150 cc per day) was routinely administered immediately after surgery and continued for 3–4 days. The other postoperative fluid therapy prior to the commencement of enteral feeding included 6% amino acids, 5% neutral fat, 10–20% glucose, and crystalloid fluid for a total of 25–30 kcal/kg/day. Seven patients had a leakage from the cervical esophagogastrostomy, which resolved after treatment with surgical dressing, and one patient died of pneumonia and sepsis within 1 week after surgery.

The preoperative workup for tumor staging consisted of esophagoscopy with biopsy, esophagogram, chest radiography, sonogram of the abdomen, computed tomography (CT) scan of the chest, and radionuclide scanning of whole body bone. All of the resected specimens were sent for histological examination. Tumor staging was classified according to the TNM system of UICC established in 1987 (14). The tumors were graded as stage I in 4 patients, stage IIa in 9, stage IIb in 4, stage III in 10, and stage IV in 10. All stage IV patients showed nodal metastasis at cervical or celiac regions. Postoperative chemoradiotherapy was administered for 24 patients with advanced stage (⩾stage IIb) lesions. The adjuvant therapy was given 1 month after surgery. The dose of irradiation was 5,000–6,000 rads (1,000 rads/week), and the combination regimens of chemotherapy consisted of 5-fluorouracil (5-FU) (600 mg/m^2/day), cisplatin (20 mg/m^2/day), and leucovorin (120 mg/m^2/day) for 4 days. Chemotherapy was concomitantly administered in the first week of irradiation.

Evaluation of Nutritional and Immune Status

The nutritional and immune status of the patients were assessed preoperatively (1 week before surgery), on the third postoperative day, and in 1 week, 2 weeks, 3 weeks, 1 month, and 3 months after surgery. One of 37 patients, who died of surgical complications on the fifth postoperative day, could not be completely evaluated.

Nutritional assessment. Biochemical studies to evaluate the patients' nutritional status consisted of studies on serum albumin, cholesterol, iron, transferrin, magnesium, zinc, total iron binding capacity (TIBC), and nitrogen balance. The serum levels of albumin, cholesterol, iron, magnesium, zinc, and transferrin were measured by an automated calorimetric technique (SMAC). Total iron binding capacity was determined by radial immunodiffusion. Nitrogen balance was calculated by measuring 24-hour urinary nitrogen excretion and 24-hour fecal nitrogen excretion by the Macro Kjedahl technique [15,16].

Immune studies. Evaluation of the patients' immune status consisted of: (1) total lymphocyte count (TLC), (2) lymphocyte subpopulation—CD3, CD4, CD8, CD20, (3) immuno-globulins (IgG, IgA, and IgM), (4) complements (C3 and C4), (5) lymphocyte blastogenic responses induced by mitogens, (6) mitogen-induced tumor necrotic factor-α (TNF-α) and interleukin-2 (IL-2) secretion activity from the patients' mononuclear cells (MNCs), and (7) C-reactive protein (CRP) level.

Assay of Igs and complements. Serum IgG, IgA, IgM, C3, and C4 were quantitated by nephelometric immunoassay using a Behring nephelometer system (Behring Diagnostics, Westwood MA).

Cell preparation. Mononuclear cells (MNCs) in heparinized blood were separated by Ficoll-Hypaque density gradient (specific gradient 1.077). The polymorphonuclear cells (PMNs) were collected from the buffy coat and were subjected to dextran sedimentation (dextran 2%, MW 50,000, at room temperature) for 30 minutes, then harvested by centrifugation. The contaminated erythrocytes were lysed by hypotonic shock. All cells were washed twice with Hanks' balanced salt solution and suspended in RPMI-1640 at 1×10^7/ml.

The MNC fraction thus prepared was composed of 85–90% lymphocytes, 10–15% monocytes, occasional PMN, and <1% basophils. The PMN fraction consisted of 90–95% PMN, 1–5% lymphocytes, 1–10% eosinophils, and <1% basophils. The viability of every cell preparation was >90% by trypan blue exclusion.

Lymphocyte blastogenic response. The culture was prepared in 96-well microtitre plates. Cell suspensions (*100 μl*), which contained 2×10^5 cells, were placed in each well. A standardized optimum concentration of phytohemagglutinin-P (PHA: Wellcome Diagnostics Dartford, UK), or concanavalin A (Con A: Sigma, St. Louis, MO), or pokeweed mitogen (PWM: Sigma) was added, and the total volume of each well was adjusted to 200 μl with RPMI 1640 medium (Gibco Laboratories, Germantown, MD). The final concentration of each mitogen was 1, 9 and 0.01 μg/ml. Wells without mitogens served as controls. The cultures were incubated at 37°C with 5% CO_2. After 72 hours, 0.5 μCi of tritiated thymidine (specific activity 6.7 Ci/mmol) was added to each well. The cultures were terminated 4 hours after tritiated thymidine incorporation by keeping the plate at 37°C. The cells were harvested onto a Whatman GF/C filter paper using a cell harvester (PHD Cell Harvester, Cambridge, MA).

After harvesting, the filter paper was transferred to a scintillation vial. Seven millilitres of scintillation mixture (1,4-bis[5-phenyl-2-oxazolyl]-benzene; 2,2′-p-phenylene-bis[5-phenyloxazole] (POPOP) 100 mg and 2,5-Diphenyloxazole (POP) 4 g per liter sulphur-free toluene) was added to each vial, and the radioactivity present in the vials was counted in a Packard, Tricarb, Liquid Scintillation Analyzer (Meridian, CT).

TNF-α ELISA and IL-2. Heparinized peripheral blood was diluted and MNC fraction was isolated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradient centrifugation. Cells in the interphase were washed in Hanks' balanced salt solution (HBSS) and suspended in RPMI-1640 supplemented with 2 mM L-glutamine, 100 IU penicillin/ml, 100 μg streptomycin/ml, and 10% heat-inactivated fetal bovine serum (FBS). Cells (2 × 106) were cultured in the presence of 5 μg/ml PHA in tissue culture tube, and incubated in 5% CO_2 at 37°C for 72 hours. Culture supernatants were then harvested and kept at −70°C until ELISA assayed for TNF-α and IL-2 (T cell diagnostics, Biokine, Cambridge, MA).

TNF-α assay. Biokine TNF test kit is a sandwich enzyme immunoassay for the determination of TNF levels in human serum, plasma, or cell culture supernatant. An anti-TNF-α monoclonal antibody was first adsorbed onto a 96-well polystyrene microtiter plate. TNF-α present in the sample or standard was bound to antibody on the coated well; unreacted sample components were removed by washing. An enzyme conjugated anti-TNF-α monoclonal antibody directed against a second epitope on the TNF molecule was then added and bound to the TNF-α captured by the first antibody to complete the sandwich. Unbound enzyme conjugated Anti-TNF-α was removed in a wash step and substrate solution was added to the wells. A colored product formed in proportion to the amount of TNF-α present in the sample. The reaction was terminated by addition of Stop Solution (1 N H_2SO_4) and absorbance at 490 nm was measured. A standard curve was prepared from five TNF-α standards. Unknown values were determined from the standard curve.

Serum TNF-α concentrations were expressed in pg/ml. One mg of recombinant TNF-α used as a standard for this assay was equivalent to 2×10^7 units of activity as defined in an L929 cytotoxicity assay in the presence of actinomycin D. One unit was defined as the amount of TNF-α sufficient to cause 50% cytotoxicity.

IL-2 assay. Biokine IL-2 test kit is a sandwich enzyme immunoassay for the determination of IL-2 levels in human serum or cell culture supernatant. A rabbit anti-IL-2 antibody was precoated onto polystyrene microtiter wells. IL-2 present in the sample or standard was bound to antibody on the coated well. Unreacted sample components were removed by washing. A monoclonal antibody to human IL-2 was then added and was bound to the IL-2 captured by the first antibody to complete the sandwich. Excess monoclonal antibody was removed by washing. An enzyme conjugated goat antimouse antibody was then added and bound to the monoclonal portion of the sandwich. After unreacted enzyme conjugate was removed in a wash step, tetramethyl benzidine (TMB) substrate solution was added to the wells. A colored product formed in proportion to the amount of IL-2 present in the sample. The reaction was terminated by addition of Stop Solution (1 N H_2SO_4) and absorbance at 450 nm was measured. A standard curve was prepared from six IL-2 standards. Unknown values were determined from the standard curve.

IL-2 concentrations were expressed in pg/ml. Recombinant IL-2 was used as a standard for this assay. Standards were calibrated to the WHO First International Standard for interleukin-2 (human) 86/504; where one WHO unit = 76 pg IL-2. For BRMP calibration 1 WHO unit = 1.9 BRMP units.

Detection of lymphocyte cell surface marker. Monoclonal antibodies for flow cytometry studies were purchased from Becton Dickinson (Mountain View, CA) and included Leu-4 (anti-CD3), Leu-3a (anti-CD4), Leu-2a (anti-CD8), B1 (anti-CD20). For controls, IgG_1 and IgG_{2a} isotypes were included. All antibodies were titered to determine optimal concentrations for staining using normal human blood MNC.

Statistical Analysis

All enrolled patients were included in the analysis; however, one patient died of pneumonia and sepsis on the fifth postoperative day. Thus the number of the study cases after the third postoperative day decreased from 37 to 36. For each variable, multiple analysis of variance for repeated measurements was used to compare the values measured before operation with the ones measured at six subsequent time points after operation. The results were presented as mean and standard error (mean ± S.E.) based on the mixed model of repeated measurements analysis. All statistical tests were two-sided and two-tailed, and the significance of *P* value was set at 0.05 in advance before study and analysis. Statistical analysis was performed with SAS software (Cary, NC).

RESULTS

The results of the nutritional and immunologic assessment at various time points are demonstrated in Table I.

Preoperative

All patients with resectable carcinoma of the esophagus were well selected and prepared before operation. As shown in Table I, their preoperative nutritional and immune status was within the assigned normal ranges.

Third Postoperative Day

The nutritional status deteriorated remarkably soon after extensive surgical resection, particularly in serum levels of iron, transferrin, cholesterol, TIBC, magnesium, and zinc ($P < 0.05$) (Table I). Similar to the nutritional deterioration by the third postoperative day, the serum levels of immunoglobulins (IgG, and A) and complements (C3 and C4) also decreased significantly (IgG, IgA, C3, C4, $P < 0.05$, respectively), along with a marked increase in CRP level (preoperation: 2.29 ± 0.82 mg/dl vs. the third day: 15.46 ± 0.94 mg/dl, $P < 0.05$). CD3 and CD8 also reduced greatly ($P < 0.05$), but CD20 increased significantly (preoperative: 7.95 ± 1.03%, vs. 3rd day: 9.52 ± 1.13%, $P < 0.05$). However, there was no statistical difference in TLC and CD4 between the results obtained preoperatively and on the third postoperative day ($P > 0.05$). The mean values of CD4/CD8 ratio, blast transformation by PHA and ConA, and IL-2 assay increased on the third postoperative day, although not to a level of statistical significance. However, a significantly higher blastogenic response induced by PWM was detected on the third postoperative day, compared to the preoperative result.

TABLE I. Nutritional and Immunity Status in Patients With Epidermoid Carcinoma of the Esophagus (Mean ± SE)†

Parameters	Preoperative (n = 37)	Postoperative 3rd day (n = 37)	1st week (n = 36)	2nd week (n = 36)	3rd week (n = 36)	1st month (n = 36)	3rd month (n = 36)
Nutrition							
Albumin (g/ml)	3.88 ± 0.09	3.06 ± 0.13*	3.65 ± 0.14**	3.61 ± 0.14**	3.71 ± 0.16	3.73 ± 0.14	3.96 ± 0.17
Cholesterol (mg/dl)	169.10 ± 8.23	85.1 ± 6.91*	89.71 ± 7.36*	121.23 ± 7.35*	140.03 ± 8.53*	145.2 ± 8.12	149.54 ± 9.23
TIBC (μg/dl)[a]	250.87 ± 13.59	131.38 ± 9.79*	157.26 ± 10.01*	188.97 ± 9.71*	197.9 ± 10.96*	200.81 ± 10.2*	218.5 ± 11.27*
Transferrin (mg/dl)	219.08 ± 11.78	101.99 ± 9.21*	128.83 ± 9.61*	173.08 ± 9.18*	181.3 ± 10.13*	187.08 ± 9.49*	214.45 ± 10.69
Iron (mg/dl)	64.83 ± 5.98	25.12 ± 5.26*	25.64 ± 5.28*	33.56 ± 5.38*	40.55 ± 5.8*	54.37 ± 5.63	63.09 ± 5.89
Magnesium (mg/dl)	2.12 ± 0.05	1.58 ± 0.06*	1.98 ± 0.06	2.21 ± 0.06**	2.09 ± 0.06	2.01 ± 0.06	1.92 ± 0.07**
Zinc (mg/dl)	106.80 ± 3.56	63.28 ± 4.46*	86.51 ± 4.41*	109.84 ± 4.58	105.29 ± 4.76	108.72 ± 4.35	88.69 ± 5.15**
24 hr UUN (mg/dl)	394.09 ± 40.06	293.31 ± 41.17**	451.82 ± 39.66	417.68 ± 42.95	454.34 ± 44.95	541.38 ± 53.16*	378.64 ± 102.94
Immunity							
IgG (mg/dl)	1216.48 ± 63.86	697.62 ± 69.9*	968.01 ± 72.72*	1352.86 ± 74.03	1434.87 ± 73.95*	1623.75 ± 71.8*	1721.45 ± 78.77*
IgA (mg/dl)	293.90 ± 24.04	188.28 ± 22.32*	262.78 ± 23.47*	322.09 ± 24.03	337.79 ± 24.23	331.31 ± 24.27	307.61 ± 25.95
IgM (mg/dl)	116.95 ± 10.43	74.6 ± 15.65**	105.38 ± 15.67	115.96 ± 16.03	149.05 ± 16.03**	134.57 ± 15.32	165.24 ± 16.86*
C3 (mg/dl)[b]	104.00 ± 4.01	72.48 ± 4.4*	95.0 ± 4.57	108.25 ± 4.66	101.2 ± 4.65	91.61 ± 4.5*	70.17 ± 4.83*
C4 (mg/dl)[b]	24.27 ± 1.22	15.77 ± 1.39*	19.67 ± 1.44*	22.52 ± 1.48	24.19 ± 1.49	27.62 ± 1.47	24.43 ± 1.56
CRP (mg/dl)[c]	2.29 ± 0.82	15.56 ± 0.94*	9.61 ± 0.97*	5.71 ± 0.99*	3.93 ± 0.99	2.61 ± 0.93	2.19 ± 1.03
Total lymphocyte count (cells/dl)	1778.79 ± 123.97	1271.44 ± 468.07	1277.86 ± 484.96	1415.12 ± 502.72	1775.42 ± 520.82	2681.99 ± 513.96	3029.03 ± 534.6**
Lymphocyte subpopulation							
CD3 (%)	60.32 ± 2.67	55.74 ± 3.15*	55.57 ± 2.95*	59.92 ± 2.97	60.86 ± 3.18	59.48 ± 2.84	60.74 ± 2.92
CD4 (%)	35.18 ± 2.04	37.31 ± 2.8	38.84 ± 2.57	36.09 ± 2.58	33.4 ± 2.8	30.37 ± 2.45*	35.96 ± 2.51
CD8 (%)	23.73 ± 1.78	20.35 ± 2.16*	18.9 ± 2.08*	23.6 ± 2.11	27.43 ± 2.22	28.4 ± 2.06	30.78 ± 2.12**
CD4/CD8 ratio	1.62 ± 0.16	2.00 ± 0.30	2.81 ± 0.62*	1.75 ± 0.16	1.55 ± 0.22	1.35 ± 0.15	1.67 ± 0.25
CD20 (%)	7.95 ± 1.03	9.52 ± 1.13*	7.25 ± 1.04	7.02 ± 1.03	7.16 ± 1.13	7.33 ± 0.99	4.85 ± 1.02**
Blastogenic responses[d]							
PHA (cpm)	9224.45 ± 1336.72	11778.6 ± 1890.6	13382.1 ± 1845.5*	11517.9 ± 1448.9	8361.2 ± 1692.5	8451.8 ± 1139.7	6620.5 ± 1146.2
PWM (cpm)	2385.18 ± 441.11	4789.34 ± 931.45*	4969.93 ± 692.66*	2623.22 ± 722.70	3425.37 ± 775.43	3355.81 ± 691.73	2301.59 ± 679.0
ConA (cpm)	6257.59 ± 1146.26	6281.03 ± 1289.03	9063.15 ± 981.31*	6732.45 ± 1021.59	5505.31 ± 1116.74	5755.4 ± 978.5	4005.1 ± 982.18
IL-2 (unit/dl)	1201.23 ± 280.65	1582.53 ± 321.04	1177.02 ± 316.94	895.51 ± 323.45	1322.36 ± 328.0	1167.18 ± 300.12	751.52 ± 307.0
TNF-α (unit/dl)	2105.23 ± 380.86	1268.42 ± 453.32**	640.76 ± 414.99*	2051.14 ± 455.26	1623.68 ± 476.67	2023.08 ± 449.91	2231.8 ± 473.22

†For each variable, multiple repeated measures analysis of variance was used to compare the values measured before operation with the ones measured at six subsequent time points after operation. One of 37 patients died of pneumonia and sepsis at the fifth postoperative day; thus the study number after the third postoperative day as decreased from 37 to 36.

[a]TIBC = total iron binding capacity.

[b]C3 and C4 = complement 3 and 4.

[c]CRP = chronic reactive protein.

[d]The blastogenic mitogens consisted of phytohemagglutin - P (PHA), concanavalin -A (ConA), and pokeweed mitogen (PWN). IL-2 = interleukin 2; TNF-α = tumor necrotic factor-α.

*$P < 0.05$; **$0.1 < P < 0.05$.

First Postoperative Week

The mean values of all the nutritional parameters were higher at the end of the first postoperative week than on the third postoperative day, particularly serum albumin, magnesium, and 24 hr urinary urea nitrogen (UUN). Compared with the preoperative results, significantly lower nutritional parameters after the first postoperative week were found in cholesterol, TIBC, transferrin, iron, and zinc ($P < 0.05$), respectively. In contrast, CRP decreased from 15.56 ± 0.94 mg/dl on the third postoperative day to 9.61 ± 0.97 mg/dl at the end of the first postoperative week, but CRP at the end of the first postoperative week was still significantly higher than its preoperative level ($P < 0.05$). The serum levels of immunoglobulins (Igs G, A, M) and complements (C3 and C4) were higher at the end of the first postoperative week than on the third postoperative day. Compared with the preoperative results in serum immunoglobulins and complements, there was no statistical difference in IgM and C3 at the end of the first postoperative week, but significantly lower levels of IgG, IgA, and C4 were observed ($P < 0.05$). TLC were still nonsignificantly lower at the end of the first postoperative week than at the preoperative stage ($P > 0.05$). For the lymphocyte subpopulation, a statistically significant difference was found in CD3, CD8, and CD4/CD8 ratio ($P < 0.05$), compared with the preoperative results. Furthermore, lymphocyte blastogenic transformation with PHA, PWM, and ConA had a significantly superior response at the end of the first postoperative week compared with the preoperative stage ($P < 0.05$). The level of TNF-α secreted from PHA-induced MNC at the end of the first postoperative week differed significantly from those at the preoperative stage ($P < 0.05$), but not the IL-2 level ($P > 0.05$).

Second Postoperative Week

The nutritional evaluation showed similar results of serum albumin, magnesium, zinc, and 24 hr UUN at the end of the second postoperative week and at the preoperative stage. Although the levels of cholesterol, TIBC, transferrin, and iron were still significantly lower than the preoperative ones ($P < 0.05$), gradual improvement was found at the end of the second postoperative week in comparison with the postoperative third day and first week. CRP further declined from 9.61 ± 0.97 mg/dl at the end of the first postoperative week to 5.71 ± 0.99 mg/dl at the end of the second postoperative week, but was still significantly higher than its preoperative level ($P < 0.05$). The serum levels of immunoglobulins (Igs G, A, M) and complements (C3 and C4) recovered progressively until the end of the second postoperative week when there was no statistical difference from the preoperative levels. Although at the end of the second postoperative week the TLC were still lower than the preoperative ones, the subpopulation of lymphocytes (CD3, CD4, and CD8) and the CD4/CD8 ratio were similar to their preoperative stage. Additionally, the lymphocyte blastogenic responses to PHA, PWM or ConA, and IL-2 and TNF-α levels decreased at the end of the second postoperative week, but the decrease did not achieve statistical significance compared with either the preoperative stage or the postoperative first week ($P > 0.05$).

Third Postoperative Week

The nutrition evaluation showed continuous improvement in the third postoperative week. However, the serum levels of cholesterol, TIBC, transferrin, and iron were still significantly lower at this time than at the preoperative stage ($P < 0.05$). The immunologic assessment showed similar results to the preoperative ones, except for IgG, which was 1434.87 ± 73.95 mg/dl, statistically higher than its preoperative value (1216.48 ± 73.86 mg/dl, $P < 0.05$).

First Postoperative Month

The nutritional results at the end of the first postoperative month were similar to those at the end of the third week, except for the serum iron level, which improved from 40.55 ± 5.8 mg/dl at the end of the third postoperative week to 54.37 ± 5.63 mg/dl at the end of the first month ($P < 0.05$). However, compared with the preoperative nutritional status, significantly lower nutritional results were found only in TIBC and transferrin ($P < 0.05$) at the end of the first postoperative month. Additionally, the patients had higher levels in serum immunoglobulins at the end of the first postoperative month than at the preoperative stage, although only the level of serum IgG was high enough to reach statistical significance ($P < 0.05$).

Third Postoperative Month

The nutritional status at the end of the third postoperative month was almost similar to the preoperative status, except for TIBC ($P < 0.05$). With regard to the immune status, IgG and IgM were in this period significantly higher than preoperatively (IgG: 1721.45 ± 78.77 mg/dl vs. 1216.48 ± 63.86 mg/dl, $P < 0.05$; IgM: 165.24 ± 16.86 mg/dl vs. 116.95 ± 10.43 mg/dl $P < 0.05$), but C3 at the end of the third month was significantly lower than the preoperative results (C3: 70.17 ± 4.83 mg/dl vs. 104.0 ± 4.01 mg/dl, $P < 0.05$).

DISCUSSION

Complete surgical resection is the most common treatment for cure of esophageal carcinoma. Unfortunately, most of these patients have dysphagia and body weight loss of various degrees to severity before the surgical operation, and a major surgical intervention as well as conventional postoperative intravenous fluid manage-

ment can induce further metabolic insult and impair the host defense.

For the patients undergoing en bloc esophagectomy with an extensive mediastinal dissection around the lung roots, removal of the thoracic duct and interruption of many lymphatics, lymph-containing fluid can be lost in huge amounts during and after the surgery. Thus a large volume of fluid replacement is inevitably required during the initial 48–72 hours after surgery [6], which may have a hemodilution effect on serum. The current investigation showed serious deterioration of all the nutritional parameters in the initial 3 days after surgery, although these patients were well selected and prepared before surgery, and intensive nutritional support was also established immediately after surgery. In the present study, the most severe degradation was found in serum iron, followed by transferrin, TIBC, cholesterol, and zinc. The mean serum levels of iron, transferrin, TIBC, cholesterol, and zinc deteriorated right after surgery to only 38%, 45%, 52%, 50%, and 59% of the preoperative status, respectively. Thereafter, the postoperative nutritional status improved gradually from the third postoperative day. Most of the parameters returned to the preoperative levels within 2–3 weeks after surgery. However, the serum levels of iron, transferrin and TIBC needed a longer period (>1 month) to return to normal. Thus an adequate supplement of iron and protein for 1–3 months after surgery is needed to prevent iron deficiency.

In the present study, the remarkable increase of serum CRP detected in the first postoperative week might indicate the possible occurrence of a severe inflammation soon after an extensive en bloc esophagectomy with esophageal reconstructive surgery. In contrast to a marked increase of serum CRP, the TLC, immunoglobulins (IgG, IgA, and IgM) and complements (C3 and C4) decreased significantly to various degrees (from 55% to 75% of the preoperative levels) in the first week after surgery, then returned gradually to the preoperative level by the second or third postoperative weeks. In contrast, CD20, which expresses most or all B-lymphocytes of total lymphocytes, reached the highest level after the third postoperative day with a gradual decline to the preoperative status ~2–3 weeks after surgery. This evidence suggests that the reduction of serum immunoglobulins and complements or the humoral immune status during the early postoperative period is possibly influenced by surgical stress and the diluting effect of the postoperative massive fluid therapy. The serum levels of immunoglobulins and complements evaluated 3 weeks after surgery were similar to, or even higher than their preoperative levels.

It has been reported that TLC, CD4/CD8 ratio, PHA response, and natural killer (NK) cell activity decrease following surgical operation [17–19], and some workers have suggested that surgical operations induce a reversible depression of the cellular immune status that precedes plasma suppressive activity in its return to the preoperative level. However, Tsutsui and his associates [20] did not find such significant reductions in the CD4/CD8 ratio, PHA response or NK activity, except that TLCs were reduced after esophageal operation. In the present study, we observed that TLCs, CD3, and CD8 diminished in the first week following the esophageal operation, whereas the CD4/CD8 ratio and lymphocyte blastogenic responses induced by each of PHA, PWM, and ConA increased. Additionally, PHA-induced IL-2 and TNF-α secretion from the patient's MNCs also increased after the third postoperative day.

Miller and his colleagues [21] have suggested that lymphopenia due to surgical stress may reflect redistribution of cells since the proportions of T and B cells remain constant. Nevertheless, our results demonstrated the occurrence of lymphopenia with a significant decrease in the relative percentage of T cells (CD3) but a contrary increase in B cells (CD20) during the early postoperative period. Although these evidences may hint at the suppression of cell-mediated immune responses after extensive surgical operation, there have been contradictory results using stimulation assays of lymphocyte function. PHA, PWM, and ConA are the three lectins frequently used in immunology as mitogens. PHA and PWM are mitogenic for both T and B lymphocytes, whereas ConA is one of the most specific T-cell mitogens. We found that the blastogenic responses induced by PHA, PWM, and ConA stimulation increased progressively after surgical resection, reached the highest level at the end of the first postoperative week, but returned to the preoperative level at the end of the second postoperative week. Wanebo et al. [22] have suggested that T-cell function is depressed by the presence of cancer and that mitogen response tests appear very sensitive to the presence of tumor and show good correlation with tumor burden. In addition, a postoperative increase in CD4/CD8 ratio presented herein suggested that esophageal resection and reconstruction results in a shift of the immunoregulatory system to helper cells. Activated $CD4^+$ T cells scan secrete cytokines, which, in turn, activate various effector cell populations. Therefore, we suggest that en bloc esophagectomy might only have a mild and temporary adverse effect on the cell-mediated immune response during the early postoperative period for patients with resectable epidermoid carcinoma of the esophagus.

It has been reported that operative manipulation of the tumor is also associated with circulating tumor cells in blood [23–25]. Thus if a down-regulation of the cellular immune response occurs in the perioperative period, the patients are at maximal risk of tumor dissemination. Lennard et al. [17] have suggested that immuno-stimulating agents such as interferon and the interleukins deserve

evaluation as preoperative prophylactic agents. However, our results revealed that PHA-induced IL-2 and TNF-α secretion from the patient's MNC did not change significantly during the perioperative period. In addition, although complete surgical resection of esophageal carcinoma is performed with the same procedure, tumor is more likely to recur in patients with multiple lymph node metastasis than in those without lymph node involvement [8–10]. Among the 36 patients of the present series, tumor recurrence was found in only two (15.4%) of 13 patients without lymph node metastasis (stages I and IIa), but 15 (65.2%) of 23 patients with lymph node involvement (stages IIb–IV) died of tumor recurrence until the date of analysis. Cell-mediated immunity may be depressed in both localized and disseminated malignancy and can be improved by successful surgical removal of the tumor [26–28]. Therefore, it may be controversial to assume that host defenses may be compromised by surgical procedures, thus providing a ''fertile soil'' for tumor cell metastasis at the very time when risks from viable tumor cells are at a maximum.

Sequential multitherapeutic modalities have been advocated as the treatment of choice for patients with esophageal carcinoma at the advanced stages [29]. Nevertheless, surgery, chemotherapy, and radiotherapy can each disturb caloric intake for an extended period of time. If the primary treatment fails to eradicate the disease, subsequent antineoplastic therapy may be compromised by malnutrition. Adequate nutritional replenishment should thus be undertaken prior to the indicated antineoplastic therapy because the malnourished patient has a much narrower safety therapeutic margin for most antineoplastic therapies. Moreover, improvement of protein-calorie malnutrition and the achievement of a positive nitrogen balance have been associated with an increase in absolute numbers of lymphocytes and T lymphocytes and a significant increase in lymphocyte response to mitogens [30]. Thus concerning the postoperative recovery of the patient nutritional and immune status, we suggest that postoperative chemoradiotherapy is optimally instituted from the third or fourth postoperative week instead of within 2 weeks of surgery particularly if the regimens of chemotherapy consist of antimetabolites such as methotrexate and 5-FU, both of which give suboptimal results in folate-deficient patients. Moertel et al. [31] have also demonstrated a significantly better response to 5-FU in patients who are nutritionally intact and active than in those who have a limited performance status.

REFERENCES

1. Wang PT, Chien KY: Surgical treatment of carcinoma of the esophagus and cardia among the Chinese. Ann Thorac Surg 1983; 35:143–151.
2. Wang LS, Huang MH, Huang BS, Chien KY: Gastric substitution for resectable carcinoma of the esophagus: An analysis of 368 cases. Ann Thorac Surg 1992;53:289–294.
3. Fahn HJ, Wang LS, Huang BS, Huang MH, Chien KY: Tumor recurrence in long-term survivors after treatment of carcinoma of the esophagus. Ann Thorac Surg 1994;57:677–681.
4. Skinner DB, Belsay RHR: Management of esophageal disease. In: ''Preoperative Evaluation, Staging, and Selection of Treatment for Esophageal Cancer.'' Philadelphia: WB Saunders, 1988, p 736–763.
5. Akiyama H: Surgery for cancer of the esophagus. ''Squamous cell Carcinoma of the Thoracic Esophagus.'' Baltimore: Williams and Wilkens, 1990, p 19–133.
6. Wang LS, Huang MS, Lin TS, Huang BS, Huang MH, Chien KY: A right thoracotomy approach with en bloc esophagectomy for carcinoma of intrathoracic esophagus—a preliminary report. J Surg Associ ROC 1991;24:829–834.
7. Wong J: Methods available to assess the quality of surgical resection. In: Giuli R (ed) ''Proceedings, First Polydisciplinary International Congress,'' OESO, Paris: Maloine, 1984, p 198–199.
8. Müller JM, Erasmi H, Stelzner M, Zieren U: Surgical therapy of esophageal carcinoma. Br J Surg 1990;77:845–857.
9. Peracchia A, Fumagalli U, Segalin A, Bonavina L: Congress issue-state of the art: Pathogenesis, diagnosis and treatment of cancer of the tubular esophagus. Diseases of the Esophagus 1985;8: 167–174.
10. Baba M, Aikou T, Yoshinaka H, et al.: Long-term results of subtotal esophagectomy with three-field lymphadenectomy for carcinoma of the thoracic esophagus. Annals Surg 1994;219:310–316.
11. Saito T, Shimoda K, Shigemitsu Y, et al.: Complications of infection and immunologic status after surgery for patients with esophageal cancer. J Surg Oncol 1991;48:21–27.
12. Saito T, Kuwahara A, Shimoda K, et al.: Enhanced immunoglobulin levels correlate with infectious complications after surgery in esophageal cancer. J Surg Oncol 1981;46:3–8.
13. Hennessy TPJ: Lymph node dissection. World J Surg 1994;18: 367–372.
14. International Union Against Cancer: TNM Classification of Malignant Tumors, rev. ed. New York: Springer-Verlag, 1987.
15. Burtis CA, Ashwood ER: ''Tietz Textbook of Clinical Chemistry.'' Philadelphia: Saunders, 1986, p 695–698.
16. Faulkner WR, Meites S: ''Selected Methods of Clinical Chemistry,'' Vol. 9. Washington, DC: AACC, 1982, p 365–373.
17. Lennard TW, Shenton BK, Borzotta A, et al.: The influence of surgical operations on components of the human immune system. Br J Surg 1985;72:771–776.
18. Lukomska B, Olszewski WL, Engeset A, Kolstad P: The effect of surgery and chemotherapy on blood NK cell activity in patients with ovarian cancer. Cancer 1983;51:465–469.
19. Hansbrough JF, Bender EM, Zapata-Sirvent R, Anderson J: Altered helper and suppressor lymphocyte populations in surgical patients. Am J Surg 1984;148:303–307.
20. Tsutsui S, Morita M, Kuwano H, et al.: Influence of preoperative treatment and surgical operation on immune function of patients with esophageal carcinoma. J Surg Oncol 1992;49:176–181.
21. Miller GC, Pritchard DJ, Ritts RE, Ivins JC, Dierre RV: Effect of surgery on the quality of lymphocyte subpopulations. JJ Surg Res 1976;21:144–148.
22. Wanebo HJ, Jun MY, Strong E, Oettgen HF: T-cell deficiency in patients with squamous cell cancer of the head and neck. Am J Surg 1975;130:445.
23. Salsbury AJ, McKinna JA, Griffiths JD, Naunton D, Morgan C: Circulating cancer cells during excision of carcinomas of the rectum and colon with high ligation of the inferior mesenteric vein. Surg Gynecol Obstet 1965;120:1266–1270.
24. Umpleby HC, Fermor B, Symes MO, Wiliamson RCN: Viability of exfoliated colorectal carcinoma cells. Br J Surg 1984;71:659–663.
25. Roberts S, Long L, Jonasson O, et al.: The isolation of cancer cells from the blood Stream during uterine curettage. Surg Gynecol Obstet 1960;111:3–11.

26. Watkins SM: The effects of surgery on lymphocyte transformation in patients with cancer. Clin Exp Immunol 1973;14:69–76.
27. Watkins SM: Cancer prognosis predicted by preoperative lymphocyte responsiveness, in vitro. Br J Surg 1976;63:433–437.
28. Haffejee AA, Angorn IM: Nutritional status and the nonspecific cellular and humoral immune response in esophageal carcinoma. Ann Surg 1978;189:475–479.
29. Wang LS, Chi KH, Hu MH, Fahn HJ, Huang MH: Management of patients with advanced T5 epidermoid carcinoma of the esophagus. J Surg Oncol 1996;62:22–29.
30. Haffejee AA, Angorn IB, Brain PP, Duursma J, Baker LW: Diminished cellular immune due to impaired nutrition in oesophageal carcinoma. Br J Surg 1978;65:480–482.
31. Moertel CG, Schutt AJ, Hahn RG, Reitemeier RJ: Effect of patient selection on results of phase II chemotherapy trials in gastrointestinal cancer. Cancer Chemother Rep 1974;58:257.